Apoptosis and Liver Injury

Hepatocytes are involved in protein synthesis, protein storage and transformation of carbohydrates, synthesis of cholesterol, bile salts and phospholipids, and detoxification, modification and excretion of exogenous and endogenous substances. Hepatocyte cell death by apoptosis is emerging as a fundamental component of virtually all acute and chronic liver diseases. The ensuing responses of cell repair, inflammation regeneration and fibrosis may all be triggered by apoptosis. Of these processes, hepatic fibrosis has the potential to be the most deleterious, as progressive fibrosis can culminate in cirrhosis.

Liver fibrosis results from chronic damage to the liver. Among the main causes are viral hepatitis (i.e. hepatitis B and C virus infection), alcoholic (ASH), and non-alcoholic steatohepatitis (NASH). An increasing body of evidence from both experimental and clinical studies suggests that hepatocyte apoptosis may contribute to liver fibrogenesis. Furthermore, recent studies in chronic HBV and HCV infection as well as non-alcoholic fatty liver disease (NAFLD) demonstrate that hepatocyte apoptosis correlates with disease activity.

Assessment of liver disease severity as well as monitoring of patients with chronic liver disease over time remains a major clinical challenge. Although liver biopsy is still considered the gold standard for assessing disease activity, it is an invasive technique with inherent risks that can be repeated only at infrequent intervals. It is poorly suited to frequent monitoring because of its expense and morbidity, and its accuracy suffers from sampling variation.

Noninvasive reliable tests to quantify the magnitude of hepatocyte apoptosis in humans, therefore, would be highly desirable, as serum biomarker for liver damage and for treatment response.

» Brochure: M30-Apoptosense ELISA - A Serum Apoptosis Marker for Chronic Liver Disease

Caspase-cleaved CK18 ("M30") as a Liver Apoptosis Biomarker

The apoptotic pathway is composed of two arms: the intrinsic pathway (initiated by cellular stress) and the extrinsic pathway (stimulated through a death receptor–mediated process). Both pathways are suspected to be involved in the pathogenesis of chronic inflammatory liver disease (CLD). In the final common step of apoptosis, the effector caspases (in particular caspase-3 and caspase-7) become activated. These specific intracellular proteases are known to cleave several cellular substrates, including cytokeratin-18 (CK18), a major intermediate filament protein expressed by hepatocytes. For selective detection of caspase-cleaved CK18, a monoclonal antibody (M30) was developed which have been shown to specifically label early apoptotic hepatocytes but not cells of the immune system. Because liver is a highly perfused organ, intracellular protein markers such as caspase-cleaved CK18 are very likely to accumulate efficiently and rapidly in the blood. These caspase-generated CK18 fragments can be detected in the blood of patients with chronic inflammatory liver disease by using PEVIVA's M30-Apoptosense ELISA. The caspase-specific cleavage product of cytokeratin 18 is in particular advantageous as CK18 is abundant in hepatocytes, thereby providing specificity for the source of this cleaved protein, and its caspase-mediated proteolytic cleavage of cytokeratin 18 is specific to cell death by apoptosis with a favourable biological half life in serum.
References:
» Mita E, et al. Role of Fas ligand in apoptosis induced by hepatitis C virus infection. Biochem Biophys Res Commun (1994) 204:468

Apoptosis Biomarker for the HCV clinic

An estimated 3% of the world’s population — more than 170 million people — are infected by the hepatitis C virus (HCV). About 70% of infections become chronic: a condition that is associated with developing end stage liver disease such as cirrhosis and hepatocellular carcinoma (HCC). In patients with chronic HCV infection M30 values largely correlate with conventional surrogate markers such as aminotransferase levels. However, patients with normal ALT and progressed HCV-related liver fibrosis were found to also have elevated caspase-cleaved cytokeratin 18 products in their serum, suggesting that M30 measurements represent an even more sensitive marker suitable for the detection of early liver injury. Serological detection of caspase activity also mirrored the degree of liver steatosis in those patients. In addition, M30 is a reliable serum marker to detect liver injury in patients with chronic HBV infection.

Even with current antiviral treatment options about 50% of HCV patients do not respond to treatment, which is costly and associated with significant side effects. There is a strong need for early detection of patients that are not responding to antiviral therapy. In a recent study it could be demonstrated that HCV patients responding to antiviral therapy revealed higher M30 serum levels prior to treatment compared to non-responding patients indicating that this blood test could be a reliable biomarker assay for the prediction of treatment response in patients with different liver diseases.

References:
» Bantel H, et al., Caspase activation correlates with the degree of inflammatory liver injury in chronic hepatitis C virus infection. Hepatology. (2001) 34:758
Apoptosis Biomarker for NAFLD Severity & NASH

Nonalcoholic fatty liver disease (NAFLD) describes a spectrum of liver abnormalities from benign steatosis to nonalcoholic steatohepatitis (NASH). In line with the observation that M30 serum levels correlate with liver steatosis in HCV-infected patients, a recent study has shown that the extent of caspase-cleaved CK18 in serum correlates with the grade of liver steatosis in patients with NAFLD. Moreover, in this study measurement of M30 serum levels allowed the discrimination between simple NAFLD and NASH and therefore, the detection of patients with increased risk of developing progressive (end stage) liver disease. This assay may therefore represent a sensitive tool for the identification of NASH patients who should be monitored more closely with respect to disease progression.

In summary, because of its high sensitivity, specificity, and positive and negative predictive value, the M30-Apoptosense ELISA has the potential to become an important instrument in clinical practice.

References: